Inter- and Intraspecific Activities of Compounds Derived from Sex Pheromone Glands of Currant Borer, Synanthedon tipuliformis (Clerck) (Lepidoptera: Sesiidae)

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Introduction

The currant borer, Synanthedon tipuliformis (Clerck) (Lepidoptera: Sesiidae) is one of the most destructive pests of the cultivated currants Ribes nigrum L. and R. rubrum L., and gooseberries, Grossularia urva-crispa (L.), in Eurasia (Manko, 1965; Yakimova, 1968; Yonghe et al., 1990; Szöcs et al., 1991; Būda, 1993; Gottwald and Künel, 1994; Karalius et al., 2003), in North America (Solomon and Dix, 1979; Szöcs et al., 1998), and in Australia (Scott and Harrison, 1978; Hardy, 1981). S. tipuliformis has one generation per year. In Lithuania, adults emerge in June. Females lay eggs on branches especially near bark wounds. About 2 weeks after hatching, caterpillars bore into the branches and develop on the starch-rich pits of currant branches. Larvae of the last stages gnaw out wide tunnels, create large pupation cells and make exit holes for emerging adults, in consequence causing the breaking of currant branches during mechanical harvesting (Hardy, 1981). In addition, microscopic fungi infest
branches through the exit holes and cause weakening of limbs. Reduced numbers of racemes per branch, fewer flowers per raceme and worse nutrition supplies lead to significant decreases of the yields of berries (Brock et al., 1964; Hardy, 1981; Vazyulya, 1982).

Biological features and life style make this pest difficult to control. The efficacy of chemical pesticides is limited by the short period when the larvae are not protected within the canes and this period often coincides with the harvest period, when pesticide use is prohibited (Grassi et al., 2002). Pheromone use as an alternative control method has been applied for monitoring and mating disruption of S. tipuliformis (Carde and Minks, 1995; Grassi et al., 2002).

The sex pheromone of this moth has been identified as a two-component mixture, consisting of (2E,13Z)-octadeca-2,13-dien-1-yl acetate (E2, Z13-18:OAc) as a major component (Szöcs et al., 1985) and (3E,13Z)-octadeca-3,13-dien-1-yl acetate (E3,Z13-18:OAc) as a minor constituent (James et al., 2001; Suckling et al., 2005). Our preliminary data indicated the presence of additional compounds, structurally related to sex pheromone components of the Sesiidae, in the extract obtained from pheromone glands of S. tipuliformis females.

The aim of this study was to determine intraspecific and interspecific activities of the compounds identified from sex pheromone glands of S. tipuliformis females.

Materials and Methods

Insects

Black currant branches containing S. tipuliformis pupae were collected at Vilnius University Botanical Garden, Kairėnai near Vilnius (East Lithuania) at the end of May 2000. The branches had been cut into pieces and the parts containing pupae had been transported to the laboratory and placed in glass containers. The temperature regime for the rearing was (22 ± 2) °C during the light part of the day and (18 ± 2) °C during the night, with the 17 h:7 h light/dark natural photoperiod. The glass containers were inspected every morning and emerged unmated adults were sexed, as it was known that high sex pheromone release activity of S. tipuliformis females started about 8 h before sunset (Buda and Karalius, 1985). Virgin females were transferred to holding containers containing a solution of 5% honey in water. In addition to moths obtained from the pupae, a number of females were collected by an entomological net in the same black currant plantation during the second half of June.

Extraction of the sex pheromone glands

When the female was found calling, her abdominal tip containing the sex pheromone gland was pushed out under mechanical pressure, excised and washed twice with 10 µl of hexane (Merck, p.a.) for 15 min. The solution was removed, concentrated to approx. 10 µl, and stored at −14 °C. In total, 12 calling females were used for this extraction.

Chemical analyses

The extract was analyzed by using the Finnigan SSQ 7000 GC-MS system, including a Varian 3400 GC instrument. Both DB-5 and DB-wax silica capillary columns (J and W Scientific, Folsom, CA, USA, 30 m, 0.25 mm i.d., film thickness 0.25 µm) were used with a temperature program of 80 °C (4 min), increased by 10 °C/min to 170 °C, then by 2 °C/min up to 210 °C and thereafter held isothermally at 210 °C for 30 min. The split/splitless injector temperature was 225 °C and the splitless period was 30 s. Helium was used as the carrier gas, with an inlet pressure of 10 psi. Electron ionization mass spectra were determined at 70 eV with the ion source at 150 °C. Mass chromatograms obtained from the sex pheromone gland extract were screened for compounds structurally related to the sex pheromone components, using diagnostic ions m/z 61 (protonated acetic acid, indicating presence of acetates), m/z 248, m/z 250 and m/z 252 ([M+−18], loss of water for octadecadienols, octadecenols and octadecanols, respectively, as well as [M+−60], loss of acetic acid for octadecadien-1-yl acetates, octadecen-1-yl acetates and octadecan-1-yl acetates, respectively). The compounds selected for analyses were identified by comparison of their mass spectral data and GC-retention times with the corresponding data from synthetic standards.

Chemicals

The synthetic compounds to be used as GC-MS standards had been obtained from Pherobank, (Wageningen, Netherlands) as well as from Flora Co. (Tartu, Estonia). The chemicals used in the
field tests had been synthesized in Tartu, Estonia, and purified by preparative liquid chromatography, as described by Mozuraitis et al. (1998). The isomeric and chemical purities of the compounds exceeded 99%.

**Field tests**

The synthetic sex pheromone components were dissolved in hexane (Merck, p.a.) and soaked from the inside into the walls of red rubber tube dispensers (8 × 15 mm). The compounds were applied either alone or in mixtures, as indicated in Tables I and II. Each lure was placed in an opaque white delta trap (trapping window sides 10 cm × 11 cm × 10 cm and trap length 18 cm), which had an exchangeable bottom (11 cm × 18 cm), coated with sticky material. (“Atracon A” traps and Pestifix glue were obtained from Flora Co., Tartu, Estonia.) Tests of the attractiveness of synthetic compounds, identified from sex pheromone gland extracts of *S. tipuliformis* females, to conspecific males were carried out in the black currant plantation at Vilnius University Botanical Garden, Kairenai near Vilnius (East Lithuania) from June 1 to 29, 2001. Each trap was fixed on a black currant branch at 3/4 of the shrub height, which is the optimal position for achieving the most abundant catches (Buda and Karalius, 1993), and was inspected and moved to the next trap location (within each replication) every 3 d. The distance between the traps was at least 15 m. Five replicates of each compound and mixture listed in Table I were used.

Bioactivity tests of synthetic compounds identified from sex pheromone gland extracts of *S. tipuliformis* females toward *S. scoliaeformis* males were conducted at the edge of a deciduous forest with birch trees dominant in Visoriai and Žemaiteliai near Vilnius (East Lithuania). Traps were fixed on branches of the shrubs growing close to birch trees about 2 m above ground and were inspected and moved to the next trap location (within each replication) every 3 d. The distance between the traps was at least 15 m. Five replicates of each compound and mixture listed in Table II were used.

**Identification of moth species**

The moths captured were identified through their external morphological characters. Representative specimens were deposited in the insect collection at the Institute of Ecology, Vilnius, Lithuania.

**Statistical analyses**

Data from the field tests were analyzed by non-parametric Kruskal-Wallis (Sokal and Rohlf, 1995) analyses of variance, followed by Mann-Whitney U-test (Sokal and Rohlf, 1995) and significantly different catches were marked with different letters at *P* < 0.05.

**Results**

**Chemical analysis of the sex pheromone glands extract**

Six compounds structurally related to sex pheromone components of clearwing moths were detected from sex pheromone gland extracts of virgin females, when GC-MS data were screened by diagnostic ions.

Compounds II and IV (Fig. 1A) showed a clear presence of the diagnostic ions *m/z* 248 and *m/z* 61, typical of octadecadien-1-yl acetates (Fig. 1B). Comparison of the retention times of natural products and synthetic standards on two capillary columns of different polarities revealed compound II as (3E,13Z)-octadeca-3,13-dien-1-yl acetate (*E*3,*Z*13-18:OAc) and compound IV as (2E,13Z)-octadeca-2,13-dien-1-yl acetate (*E*2,*Z*13-18:OAc). Fragmentation patterns of mass spectra of *E*3,*Z*13-18:OAc and *E*2,*Z*13-18:OAc, recorded from the extracts, corresponded well to the ones of synthetic standards.

Compound VI showed a mass spectrum that was very similar to those of II and IV. The complete absence of diagnostic ions at *m/z* 61 (Fig. 1B) suggested that compound VI was octadecadienol. Synthetic (2E,13Z)-octadeca-2,13-dien-1-ol (*E*2,*Z*13-18:OH) had the same retention time as compound VI and the two compounds showed identical mass spectra.

The occurrence of diagnostic ions at *m/z* 250 in mass spectra obtained from compounds I and V as well as the presence of *m/z* 61 only in the mass spectrum of compound I indicated that the natural products I and V were octadecen-1-yl acetate and octadecenol, respectively (Fig. 1B). The stereochemistry and the position of the double bond in both compounds were determined as *Z*13-, by comparison of retention times of natural products and corresponding characteristics of synthetic
standards, indicating that compounds I and V were (13Z)-octadec-13-en-1-yl acetate (Z13-18:OAc) and (13Z)-octadec-13-en-1-ol (Z13-18:OH), respectively.

Compound III showed a mass spectrum that was very similar to that of octadecan-1-ol (18:OH), presented by the Mass Spectral Library, version 1.7 of National Institute of Standard and Technology, USA. Comparisons of the mass spectra and retention times of the natural product and the ones of the synthetic standard confirmed that compound III was octadecan-1-ol.


Field trapping

Bioassay tests of synthetic compounds, identified from sex pheromone gland extracts of virgin S. tipuliformis females, revealed that only the main component E2,Z13-18:OAc was attractive to conspecific males, when two sex pheromone components were tested alone under field conditions (Table I). Addition of E3,Z13-18:OAc to the main component increased the effectiveness of E2,Z13-
18:OAc over seven times. The attractiveness of six component lures did not differ significantly from the one of the binary lures. In conclusion, E3,Z13-18:OAc was the synergist of E2,Z13-18:OAc and these two acetates in the ratio 0.7:10 were essential sex pheromone components of S. tipuliformis.

E2,Z13-18:OAc, tested alone, was also attractive to the males of another Synanthedon species, S. scoliaeformis. The presence of E3,Z13-18:OAc in the binary mixture with E2,Z13-18:OAc suppressed the attraction of S. scoliaeformis males 2.5 times. However, due to small catches of moths in the black currant field, the antagonistic effect was not statistically significant (Table I).

The trapping tests, carried out at the dwelling place of S. scoliaeformis, revealed that E3,Z13-18:OAc, when present in the binary mixture with E2,Z13-18:OAc, suppressed the attractiveness of the latter acetate entirely (Table I). Other compounds identified from sex pheromone glands of S. tipuliformis did not have a significant effect on the attraction of S. scoliaeformis males.

Thus, in addition to the intraspecific synergistic effect, E3,Z13-18:OAc increased the specificity of the pheromone signal for S. tipuliformis, when acting by interspecific way as an attraction antagonist to S. scoliaeformis males.

**Discussion**

GC-MS analyses of crude pheromone gland extracts demonstrated that virgin S. tipuliformis females produced E2,Z13-18:OAc, E3,Z13-18:OAc, Z13-18:OAc, E2,Z13-18:OH, Z13-18:OH and 18:OH in the ratio 100:0.7:2.7:3:traces:traces. The first 3 compounds were previously known to
occur in the sex pheromone gland extracts, while the last 3 chemicals were reported for the first time.

The attractiveness of E2,Z13-18:OAc to S. tipuliformis males was discovered by Voerman et al. (1984). Two years later, Priesner et al. (1986) reported, that the attractiveness of E2,Z13-18:OAc was significantly enhanced by the positional isomer E3,Z13-18:OAc, when they were tested together at the ratios 100:3 and 100:10. Later research on synergistic effects of E3,Z13-18:OAc revealed that two strains of currant borers exist according to the response of males to a two-component attractant. Males of the first strain occurring only in Tasmania were clearly attracted to E2,Z13-18:OAc as a single compound (Szőcs et al., 1990; Suckling et al., 2005), whereas males of the second strain significantly preferred a two-component blend, consisting of E2,Z13-18:OAc and 3% E3,Z13-18:OAc. The second strain was found in Europe (Voerman et al., 1984; Szőcs et al., 1990, 1991), New Zealand (Szőcs et al., 1990) and North America (Szőcs et al., 1998). Our trapping data confirmed the synergistic effect of E3,Z13-18:OAc on E2,Z13-18:OAc which was expected to occur for the European population of currant borers.

Shortly thereafter the sex attractant was reported: two compounds, E2,Z13-18:OAc and Z13-18:OAc in the ratio 97:3, were identified from sex pheromone gland extracts by Szőcs et al. (1985) and only the dienic acetate was confirmed as the sex pheromone. A minor sex pheromone component, E3,Z13-18:OAc, in trace amounts, was detected by means of the GC-EAG method from sex pheromone gland extracts of single females (James et al., 2001). Suckling et al. (2005) have demonstrated that in New Zealand sex pheromone components occurred at the ratio 97:3. We have found that in sex pheromone gland extracts, obtained from currant borer females of Lithuanian population, E2,Z13-18:OAc and E3,Z13-18:OAc were present in the ratio 100:0.7 which is somewhat higher than 100:3 that was used in the optimized lures reported by Szőcs et al. (1990) and identified from sex pheromone gland extracts of New Zealand population (Suckling et al., 2005).

The compositions of sex pheromones and attractants among Sesiidae are highly conserved as, to date, the E,Z- and Z,Z-isomers of 3,13- and 2,13-octadecadienols, the corresponding acetates and (2E,13Z)-octadeca-2,13-dienal are used in sexual communication by clearing species [http://www.pherobase.com, an updated website based on the book of Arn et al. (1992)]. Consequently, numbers of Sesiidae species use the same compound as the main sex attractant component, and the specificity of a sex attraction signal could be achieved due to minor components with inter- or/and intraspecific activity. Buda et al. (1993) demonstrated that two related Synanthedon species, S. tipuliformis and S. scoliaeformis, used E2,Z13-18:OAc as their main attractant component. Our data revealed that E3,Z13-18:OAc showed a dual behavioural activity, by synergising attractiveness of E2,Z13-18:OAc to S. tipuliformis males, and acting as an attraction antagonist to males of the S. scoliaeformis species, by this way ensuring the specificity of the sex attraction signal of the currant borer.

In addition to the sex pheromone compounds of S. tipuliformis, another 4 chemicals E2,Z13-18:OH, Z13-18:OAc, Z13-18:OH and 18:OH, identified from the sex pheromone glands, did not show any biological activity, neither to S. tipuliformis nor to S. scoliaeformis males. However, they provide a basis to achieve specificity of a pheromone signal of S. tipuliformis and could act as attraction antagonists against other clearing moth species which, like S. tipuliformis, employ E2,Z13-18:OAc as their sex pheromone component. Some of these compounds could be intermediates or side products in biosynthesis of a sex pheromone as well.

It is known that moths from one and seven species of the families Cossidae and Sesiidae, respectively, were attracted to single E2,Z13-18:OAc (based on http://www.pherobase.com). Consequently, the two compounds E2,Z13-18:OAc and E3,Z13-18:OAc have to be present in pheromone formulations used either for monitoring or for control of S. tipuliformis to avoid effects on non-target species.

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Yonghe Z., Ruxian B., Wendong L., and Xin Z. (1990), The occurrence of Synanthedon tipuliformis (Clerck) and its control. Insect Knowledge 27, 148–149.